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Washington DC 20231

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 C.F.R. 1.53 (c).

Docket Number	110.0210 0160	Type a plus sign (+) inside this box >	+

INVENTOR(s)/APPLICANT(s)

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TITLE OF THE INVENTION (280 characters max)

PLANAR WAVEGUIDE DETECTION SYSTEM AND METHOD

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ENCLOSED APPLICATION PARTS (check all that apply)

X	Specification	Number of Pages	30	____	Small Entity Statement
____	Drawing(s)	Number of Sheets	____	____	Other (specify)

METHOD OF PAYMENT (check one)

X	A check or money order is enclosed to cover the Provisional filing fees	PROVISIONAL FILING FEE AMOUNT	($) 160.00
X	The Commissioner is hereby authorized to charge any additional required fees or credit overpayment to Deposit Account Number: 13-4895		

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government:

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____ Yes, the name of the U.S. Government agency and the Government contract number are: ____

Respectfully submitted,

SIGNATURE KW Raasch Date 13 NOVEMBER 2002

TYPED OR PRINTED NAME Kevin W. Raasch REGISTRATION NO. 35,651

____ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

Provisional Cover Sheet.wpd Rev 000929

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): WULFMAN et al.

Docket No.: 110.0210 0160

Title: PLANAR WAVEGUIDE DETECTION SYSTEM AND METHOD

Assistant Commissioner for Patents
ATTN: **BOX PROVISIONAL APPLICATION**
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We are transmitting the following documents along with this Transmittal Sheet (which is submitted in triplicate):

X PROVISIONAL PATENT APPLICATION including:
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- ___ Drawings (___ figures on ___ sheets);
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X A check in the amount of $ 160.00 to pay the provisional application filing fee.
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PATENT
Docket No. 110.02100160

PLANAR WAVEGUIDE DETECTION SYSTEM AND METHOD

The present invention relates to the field of detection systems and methods. More particularly, the present invention provides a planar waveguide assay detection system and methods of using the same.

Assays for detecting and/or identifying a wide variety of analytes are known. In many instances, the assays are designed in an array format with a surface on which the analyte (e.g., DNA, oligonucleotides, etc.) is bound to one or more specific sites. Detection can then be performed, for fluorescent detection systems, by providing excitation energy that causes the sites binding the analyte to fluoresce. Obtaining and processing an image of the array can then provide identification of those sites at which the analyte is bound to identify, confirm the presence of, and/or determine a concentration of the analyte.

Other detection systems and methods such as those described in U.S. Patent No. 5,843,651 (Stimpson et al.) also rely on binding of analytes to the surface of an array in the form of a waveguide. Rather than relying on fluorescent excitation, however, Stimpson et al. detect light scattered at the surface of a waveguide as a result of illuminating the entire waveguide through an edge thereof.

Conventional fluorescent detection systems using waveguides involve the use of a stationary excitation source that delivers excitation energy in the form of an evanescent wave to an entire surface of the waveguide at the same time. In other words, the excitation energy is not selectively delivered to discrete areas of the waveguide. As a result, the detection methods are typically limited to developing an image of the entire array surface simultaneously or scanning the array surface to develop an image thereof.

The present invention provides a planar waveguide detection system and methods of using the same. The system includes a planar waveguide with first and second opposing major surfaces and an array with at least one and preferably a plurality of binding sites located on the first major surface of the planar waveguide. An excitation source is mounted to deliver excitation energy in a direction generally normal to the second major surface of the waveguide, such that the excitation travels through the thickness of the waveguide towards the first major surface and the binding site or sites located thereon. Fluorescent signal energy emitted from one or more of the binding sites in response to the excitation energy enters the first major surface of the waveguide and propagates within the waveguide by TIR until it reaches an edge of the waveguide. A detector is located proximate the edge of the waveguide to detect the fluorescent signal energy.

The excitation source is provided in a manner such that the excitation energy emitted from the source scans or moves over the second major surface of the waveguide. For example, one or both of the excitation source and the waveguide may be physically moved relative to each other to provide the desired scanning motion. Alternatively, the excitation energy may be scanned across the second major surface of the waveguide optically, e.g., using mirrors, optical fibers, waveguides, etc.

The detector may be operably connected to a controller provided to process the signals generated by the detector in response to the detection of fluorescent signal energy from the planar waveguide. The signal energy may be processed based on a variety of parameters such as wavelength, signal strength, etc. Furthermore, the detector signals may be correlated to the position of the scanning excitation energy on the planar waveguide to establish the location of the binding site emitting the detected fluorescent energy.

It may be preferred that the binding sites on the array be spaced apart from each other along the direction in which the excitation is scanned across the waveguide. Multiple detectors may be positioned along the edge of the waveguide to detect fluorescent signal energy from a plurality of binding sites simultaneously.

The attached documents provide descriptions of illustrative systems and methods according to the present invention on pages A-1 through A-18. Pages A-19 through A-21 set forth one deconvolution strategy for deconvolving a detector signal according to the methods of the present invention. Pages A-22 through A-25 depict results from a variety of experiments conducted using the systems and methods of the present invention. Pages A-26 through A-27 list various aspects of the present invention.

All patents, patent applications, and publications cited herein and/or in the attached documents are each incorporated herein by reference in their entirety, as if individually incorporated by reference. Various modifications and alterations of this invention will become apparent to those skilled in the art without departing from the scope of this invention, and it should be understood that this invention is not to be unduly limited to the illustrative embodiments set forth herein.

Introduction

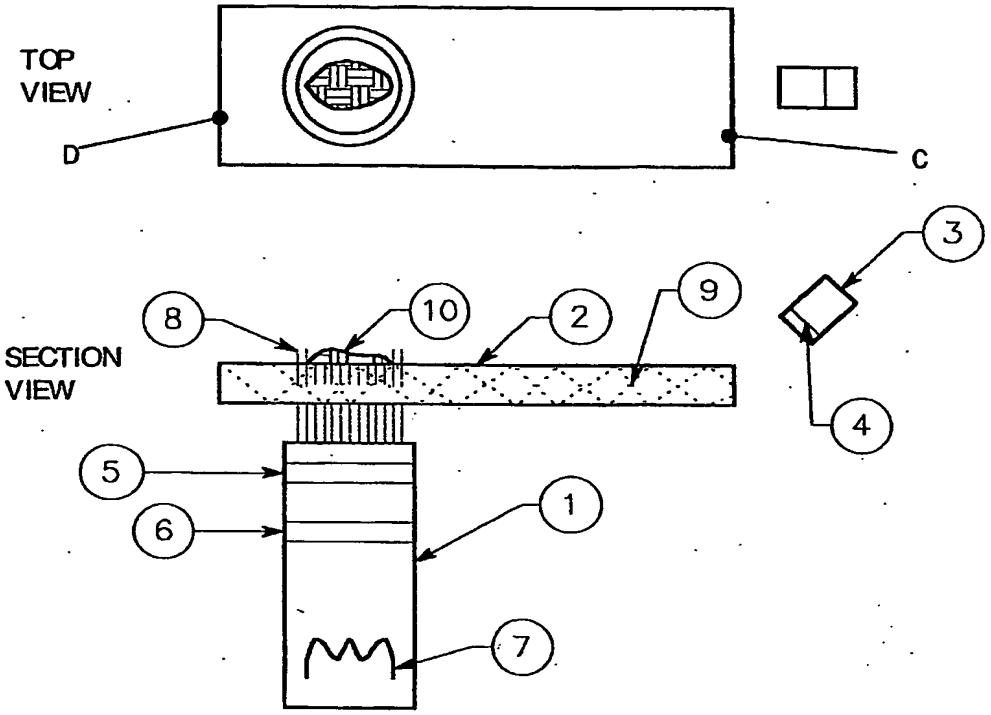
This document describes a system for executing molecular diagnostic assays, employing fluorescent markers to read results. Although the system as a whole combines the means to collect, purify, amplify, and detect specific DNA sequences, the detection part of the system is the primary focus here. A brief overview of the whole is followed by specific description of different detection means along with variations.

System Overview

The general system is composed of the following elements organized by function.

1. Collection: this component serves to receive a sample (e.g., blood) minimizing the chance of damaging stress, or loss of the sample, delivering it to the proper location in the system for appropriate preparation and analysis.
2. Purification: removes unnecessary sample components that could otherwise inhibit later processes.
3. Amplification / Hybridization: through one or many reactions taking place in the reaction chamber (e.g., PCR, LDR, and Invader) a fluorescently labeled oligonucleotide target to may be activated if a specific DNA sequence in the tissue sample is present. This target is designed to hybridize to a probe. On a surface of the reaction chamber (probe surface) are attached oligonucleotide probes whose free ends are made up of the complementary sequence to one of the ends of the activated fluorescently labeled target. If the activated targets are present, they hybridize to the probes making the probe surface fluorescent. This process is regulated by the addition of chemical reagents in a thermally controlled aqueous environment. Because immobilization of probe is coordinate specific, multiple DNA sequences may be simultaneously queried within the same reaction chamber.
4. Detection: unlike most common detection methods currently employed, which rely on pattern recognition of an image taken of a DNA or oligonucleotide microarray, or liquid wells, the detection schemes described here take a different approach. The schemes disclosed here in their most basic form involve exposing the probe surfaces to the appropriate wavelength light (excitation light), which the fluors absorb. The fluors then re-emit the light at a longer wave length (emission light), which is then detected by a properly tuned photo detector. If the photo detector detects emission from a given probe surface, then it is inferred that the DNA sequence associated with its complimentary marker is present in the sample.

The remainder of this document describes several methods and means of detection using a planar wave guide as a probe surface.



No.	Key	Function
1	Light Source	Provides photo excitation
2	Planar Wave Guide (PWG)	Sample substrate & carrier for emission light
3	Photo Detector	Detects light emitted from sample
4	Emission Filter	Filter that allows only emission wave length to pass
5	Optics	Collimates light emitted from lamp
6	Excitation Filter	Filter that allows only excitation wave length to pass
7	Lamp	Produces light
8	Excitation Rays	
9	Emission Rays	
10	Probe Surface	Target Hybridization for later detection

Figure 1: Planar Wave Guide Detection Layout 1

Figure 1 shows the basic layout of a planar wave guide detection system. It is composed of a planar wave guide (PWG) (2) aligned to both an excitation light source (1) and a photo detector (3). On the PWG (2) probes are immobilized (10) that can hybridize to a

specific DNA target sequence. The immobilized probe coincides directly with the light source output. The light source (1) is composed of a lamp (7), excitation filter (6), and optics (5). The photo detector (3) is configured with an emission filter (4) and is aligned with the PWG (2) so as to receive the maximum light that might emit from it. A photo detector could include a photodiode or a photo multiplier tube.

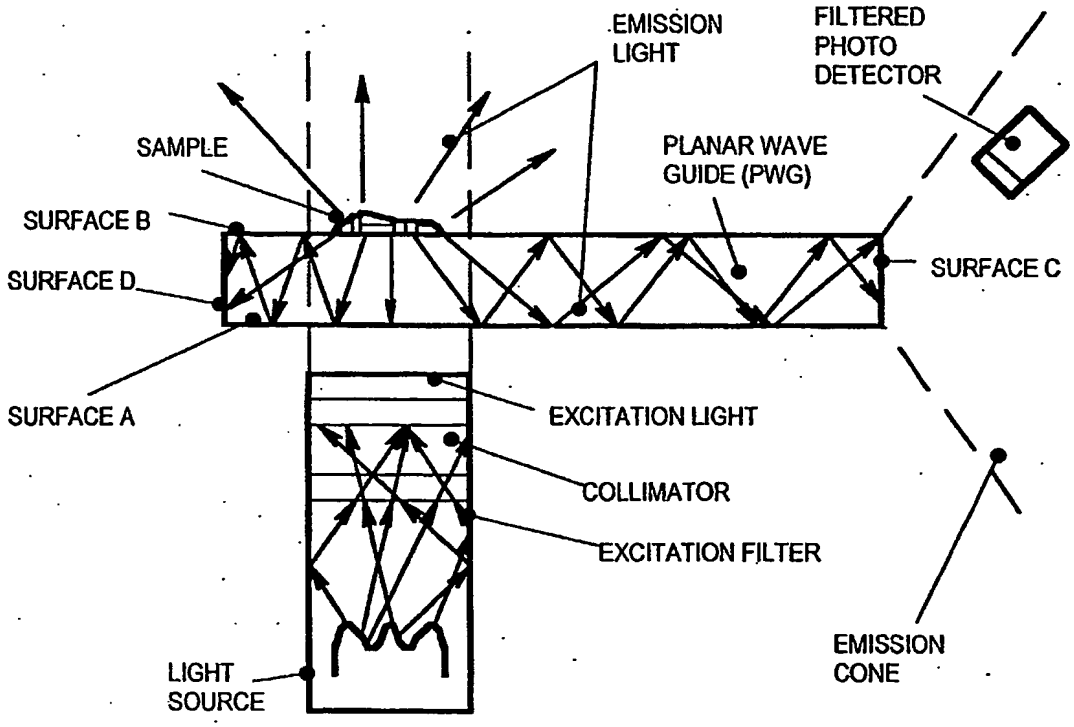


Figure 2: Light Path Illustration

Figure 2 illustrates the functionality of the system. Generally, a light source is positioned to coincide with the probe surface located on surface B of the PWG. The excitation light emitted from the light source strikes the lower surface A of the PWG and travels through the PWG, striking the probe surface B. If the fluorescent target is present on the probe surface, it absorbs the excitation light and re-emits light at a longer wave length. This emission light travels through the PWG exiting out of its edges, including surface C. A

portion of the emission light leaving surface C, strikes the filtered photo detector, which in turn transduces the light into an electric current. If the detector produces an electric signal, then it may be inferred that the fluorescent target has hybridized to the probe surface B of the PWG and further inferred that the DNA sequence associated with the fluorescent target is present in the sample.

In more specific terms, the light source produces a dispersed pattern of white light, that first travels through an excitation filter allowing only a band to pass through it, within which is the wave length that excites the selected fluorescent label. The band of light allowed to pass, then travels through collimating optics, producing rays of light that are perpendicular to the PWG surfaces A and B. This collimating ensures that the majority of the light that actually enters the PWG, passes through it and strikes the sample, instead of propagating through internal refraction to other locations in the wave guide.[1] This is advantageous because it maximizes the excitation of the sample for a given light source, and minimizes the noise that propagates through the PWG.

If the probe surface has fluorescently labeled targets hybridized to it, the fluors absorb the excitation light and then re-emit at their emission wave length. As figure 2 shows, the fluors re-emit in a scattered pattern, some of which travels into the surrounding medium and is lost, while the rest enters the PWG. Of the portion of light entering the PWG some will have such an angle of incidence on surface A, that it passes through surface A and also escapes into the surrounding medium. The remaining portion is trapped inside the PWG by internal refraction, and propagates through it in a net direction parallel to surfaces A and B. A portion of the propagated light exits surface C where it can be detected by the photo detector.

Conversely if no fluor labels are present on the probe surface, then no emission light will propagate through the PWG, and the photo detector will not be excited. In this way, the presence or absence of hybridized target can be queried without the need for imaging. In current fluorometric detection methods, imaging is a common means employed for confirming the presence or absence of DNA sequences in a sample. These imaging methods require either a person or automated system to read and interpret these images, which is not required in the implementation of the system(s) disclosed here.

[1] It should be noted that although excitation and emission filters are shown here, given the proper photo isolation between the probe surface and the photo detector, they could be eliminated. This is possible because of the collimating of the excitation light. Since the collimated excitation light enters the PWG perpendicular to surfaces A and B, the majority of it will pass directly through the PWG and out surface B. The rays coincident with the probe surface, will be absorbed by it and either be re-emitted in a dispersed pattern as heat or as the emission wavelength associated with the fluorochrome label of the target hybridized to it. This leaves very little, if any, of the excitation light to actually propagate through the PWG and excite the photo detector. That is to say, whatever light actually excites the photo detector, if any, comes from emission of the fluorochromes and not from the light source. In effect, the combination of collimating, orientation, and the geometry of the PWG, serves as a filtration system itself.

Variations

Focusing

Although the top view of Figure 1 shows the PWG as a rectangle, the actual shape of the surfaces A and B can be configured such that the light exiting surface C is more focused and intense. As illustrated in Figure 3, the long walls of the PWG can converge making surface C narrower than surface D. If the angle of convergence is small enough to effect total internal reflection, then the light propagating toward surface C is condensed. The same flux of light exits through surface C in Figures 1 and 3, but because surface area shown in Figure 3 is smaller, its light is more intense. With the appropriate placement of a small photo detector (like a photodiode), a greater per cent of the actual light emitted from the fluors is channeled for detection. The light rays shown in Figure 3 are depicted for the purpose of concept illustration, and do not depict the total pattern of light dispersed from the probe surface shown.

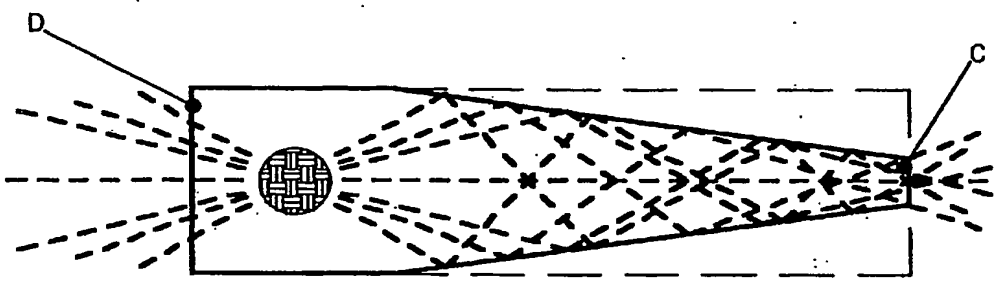


Figure 3: Focusing PWG

Multiplexing

Multiplexing is defined here as detecting multiple DNA sequences from the same sample using multiple probe surfaces. This may be accomplished through a number of means. Two different means will be illustrated here. The first involves packaging separate planar wave guides together such that their associated probe surfaces share the same reaction chamber, yet have sufficient photo isolation from one another that they do not produce crosstalk during detection. The other means involves placing multiple probe surfaces on the same planar wave guide, which has been configured to accomplish the same photo isolation as in the first means.

Common Themes

Both of the multiplexing systems shown in Figures 4 and 6 share a hierarchy of apparatus. There is either one or a series of planar wave guides mounted in a structure that provides support, protection, and alignment for the wave guide(s). The wave

guide(s) and structure combine in such a way that they also create a vessel in which the biological sample is prepared for detection. This vessel is known as the reaction chamber. This preparation may include all or some of the processes of purification, amplification, and hybridization. This combined wave guide and support-structure assembly, the Reaction Chamber Assembly (RCA) is disposable. It is used only once for the analysis of a single sample.

A second apparatus, the Reaction Controller/Detector (RCD) is a reusable device, in which are located the appropriate controlling circuitry for the reaction chamber, light source(s) and detector(s), as well as any of the power sources to enact the reaction or detection processes. The RCD is configured to mate with the RCA in such a manner that probes are easily aligned with light source(s) and photo detectors with outputs of PWG(s).

Multiplexing Means 1

Figure 4 illustrates a possible configuration that accomplishes the goals of multiplexing. The floor of the reaction chamber can be characterized as a series of glass plates held together in an opaque plastic matrix. The plates are configured such that one end serves as a portion of the reaction chamber floor, while the other extends outside the chamber, where it can transmit any emission light to a photo detector.

An individual glass plate bears a specific probe associated with a given DNA sequence. If that sequence is present in the sample and the associated complimentary fluorescent target hybridizes to it, then the fluor will produce emission light when exposed to the proper excitation light. Since the plate is optically isolated from the other plates making up the rest of the reaction chamber floor, it is the only plate through which emission light emanating from its probe surface travels. Further, because of optical isolation, no other emission light from a probe surface of other plates has the access to travel through it. This kind of isolation permits the separate detection of different DNA sequences from the same sample within the same chamber.

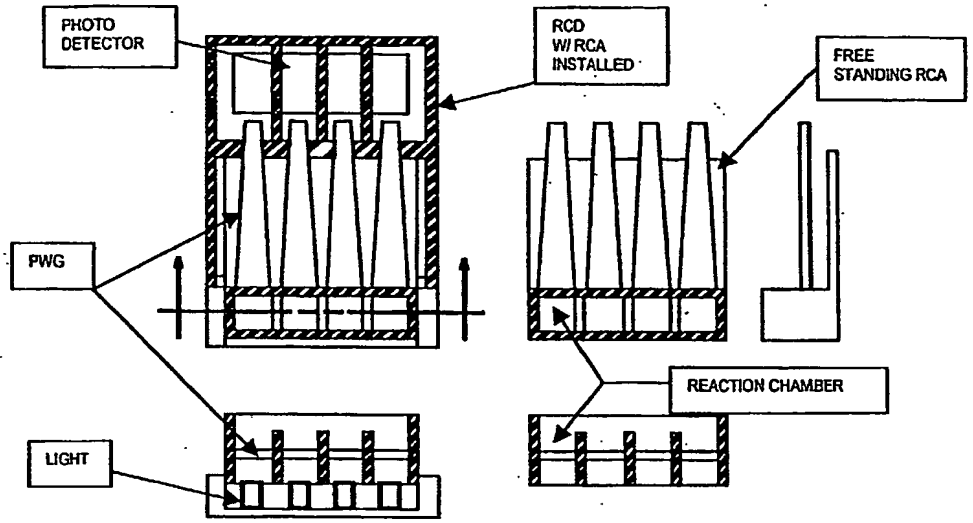


Figure 4: Multiplexing Means 1 illustration

Excitation may be accomplished either by illuminating the entire floor of the chamber with a broad beam excitation source or illuminating individual plates with a more focused source. Detection is achieved through the use of a photo detector like a photodiode or a photo multiplier tube. A single photo detector may be employed to sample the entire device by sequentially aligning it to each individual output surface (like surface C shown in Figures 1 and 2) and sampling for light. Multiple photo detectors, each aligned to a different plate output surface may also be used to reduce the mechanical complexity of the system by minimizing the number of moving parts, and to afford simultaneous sampling of multiple probe/targets.

Photo-isolation between PWGs is illustrated in greater detail in Figure 5. Whether illuminated separately as shown, or by a larger common light source, each of the probe surfaces is optically isolated from the others by means of the partial height walls shown in section B-B of Figure 5. Since the walls do not extend above the liquid level of the sample mixture, the probe surfaces share a common fluid source.

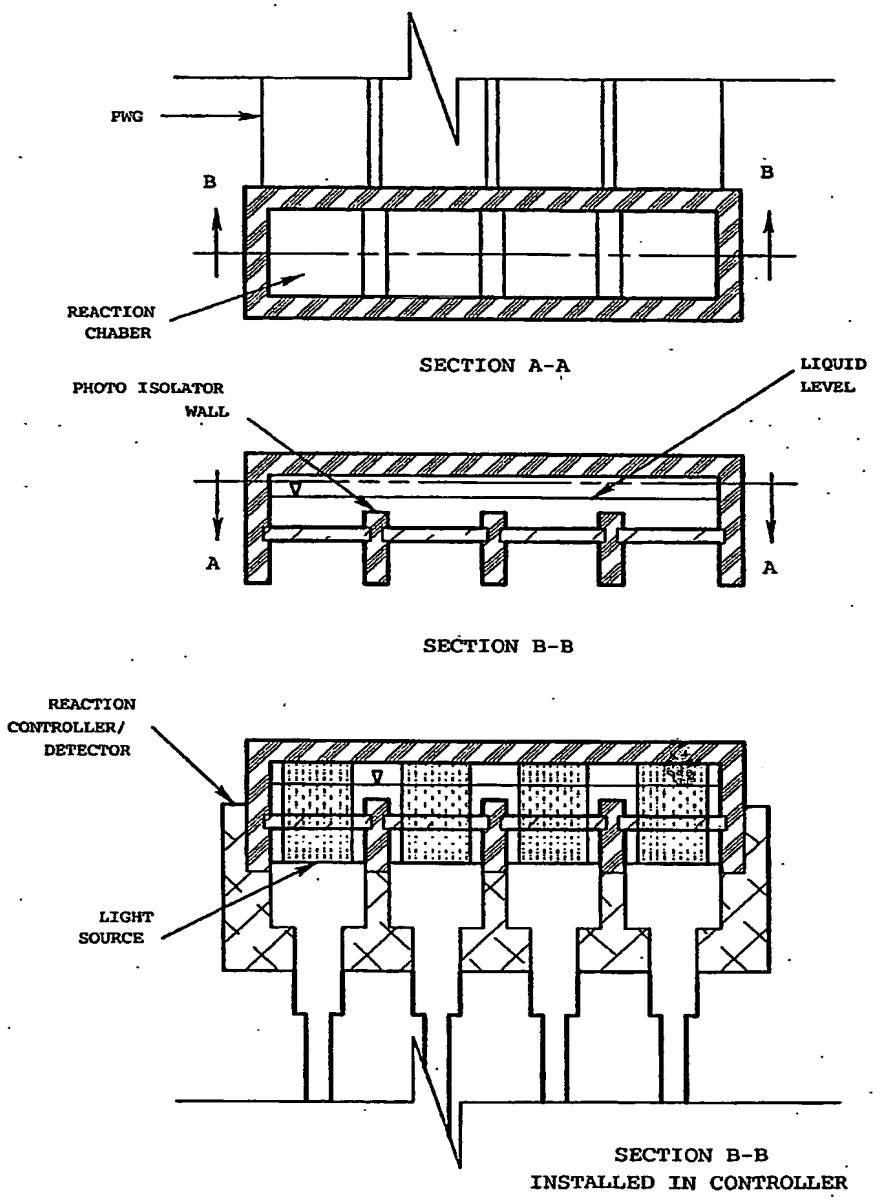


Figure 5: Photo Isolation Illustration

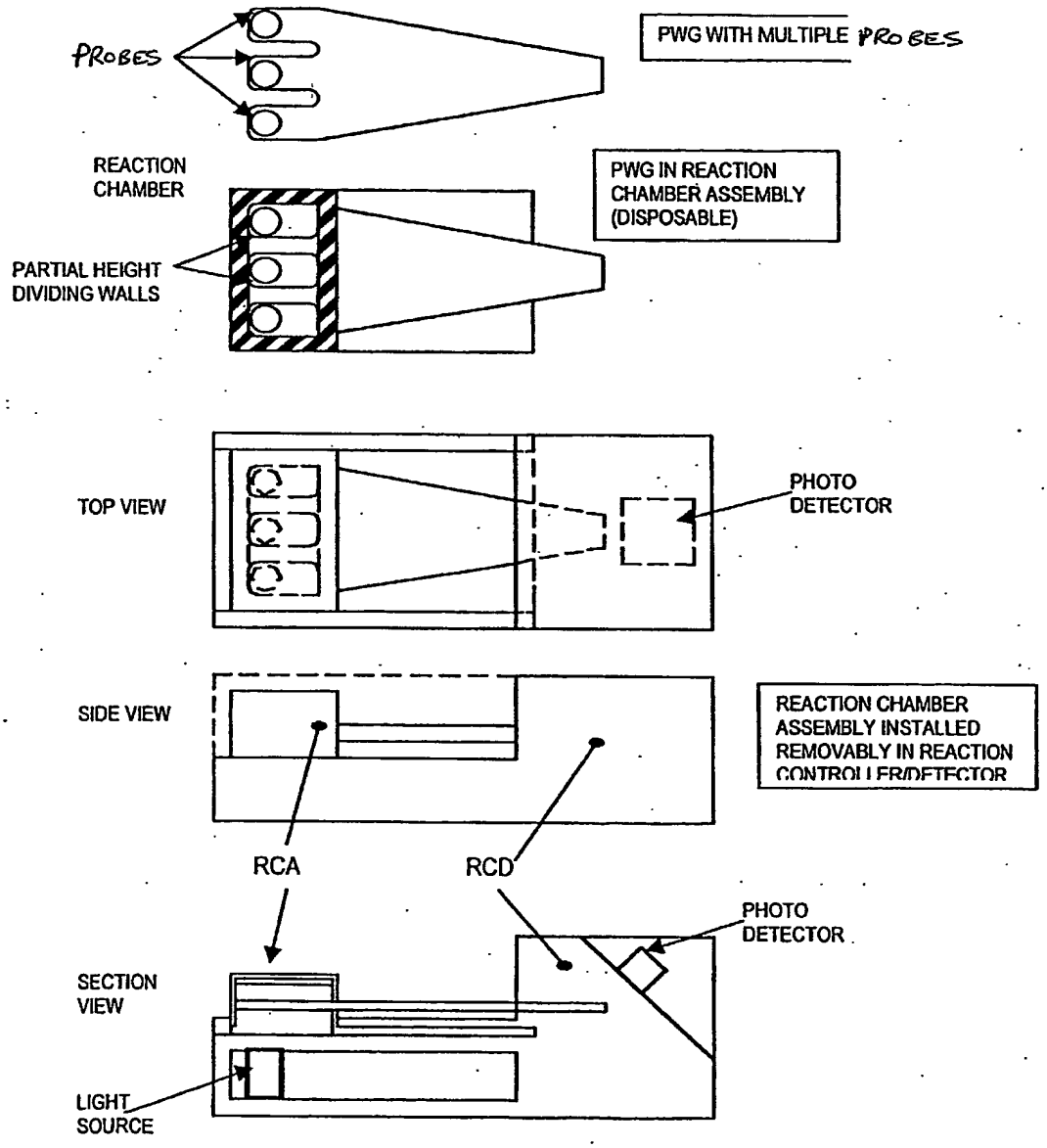


Figure 6: Multiplexing Means 2 Illustration

Multiplexing Means 2

Figure 6 demonstrates the second means of multiplexing. In this strategy a single glass plate is employed, at one end of which is a series of protruding teeth, similar to those of a saw. When integrated in the reaction chamber assembly, the teeth are separated from one another photonically, but connected together fluidically. The opposite end of the glass plate is aligned to a photo detector. To detect for fluorescence, each tooth is individually and sequentially illuminated by collimated excitation light source(s). Due to the above described configuration, only one tooth is excited at a time, providing the means to differentiate. If it is known which tooth is being illuminated at a given moment, then the presence or absence of signal in the photo detector at that same moment indicates the presence or absence of the DNA sequence in the sample that is associated with the probe immobilized to that tooth.

Figure 6 shows one method of photonically separating the different teeth while maintaining a fluidically continuous space. Similar to Multiplexing Means 1, the teeth of the plate make up the floor of the reaction chamber. In between the teeth are constructed walls of opaque material projecting above the tooth surface into the chamber. These walls function as light blinds, photonically isolating the teeth from one another. The walls do not project to the top of the reaction chamber, allowing liquid to flow over them, thus keeping the teeth fluidically connected.

Concluding Remarks

A general means of fluorescently detecting gene sequences using a planar wave guide (PWG detection) has been discussed. The functional principles of PWG detection have been presented and are in the process of further development and testing. Following the general discussion, two methods of multiplexing the PWG detection concept have been presented demonstrating how each is implemented. The first is composed of multiple wave guides arranged within an optically opaque matrix, the second, a single wave guide within which multiple optically isolated probe surfaces are integrated.

Addendum 1

Introduction

This document is an addendum to the disclosure of pages A1-A10, referred to herein as the original document. Below is presented another embodiment of the invention described in this initial document where a fluorescent detection means is disclosed employing the optical transmission properties of a planar wave guide. Unlike the methods described in the initial disclosure, which include, e.g., multiple discrete surfaces for multiplexing, this embodiment requires one surface on which a known pattern of oligonucleotide spots is placed. Reducing the detection system to a single wave guide surface affords many opportunities to reduce the complexity, scale, and part count of a given sensing system.

General Detection Process

A single surface is prepared with a known pattern of oligonucleotide spots (probe spots) spaced appropriately far apart from one another such that their dual fluorescent signals are distinguishable from one another (Refer to the signal reading section for more discussion on spot distinction). As described in the preceding document, each spot is composed of unique oligonucleotide probes whose fluorochrome labeled compliments are associated with different genetic sequences sought in a sample. If a target sequence is present in a sample, the fluorochrome labeled compliment is liberated into solution. Through mixing and diffusion kinetics, the compliment encounters the immobilized oligonucleotide and hybridizes to it. Since a given probe molecule is present in one or a given set of spots on the substrate, only the probe spots whose fluorochrome labeled complements are present in solution will become fluorescently excitable. In this application, the surface upon which probes are immobilized is that of an optical waveguide, composed of glass or some other medium that permits transmission of electromagnetic energy at wavelengths consistent with those associated with the excitation and emission of the fluorochrome(s) in use.

The level of emission from a given spot is linearly dependent upon the intensity of excitation light to which it is exposed. In turn the intensity of a spot's excitation is effected by both the initial intensity of the excitation light, and the degree of alignment between the excitation light spot and the probe spot. Initial test data suggests that fluorescence from a given probe spot varies inversely with the square of the offset distance between the probe spot and light spot centers.

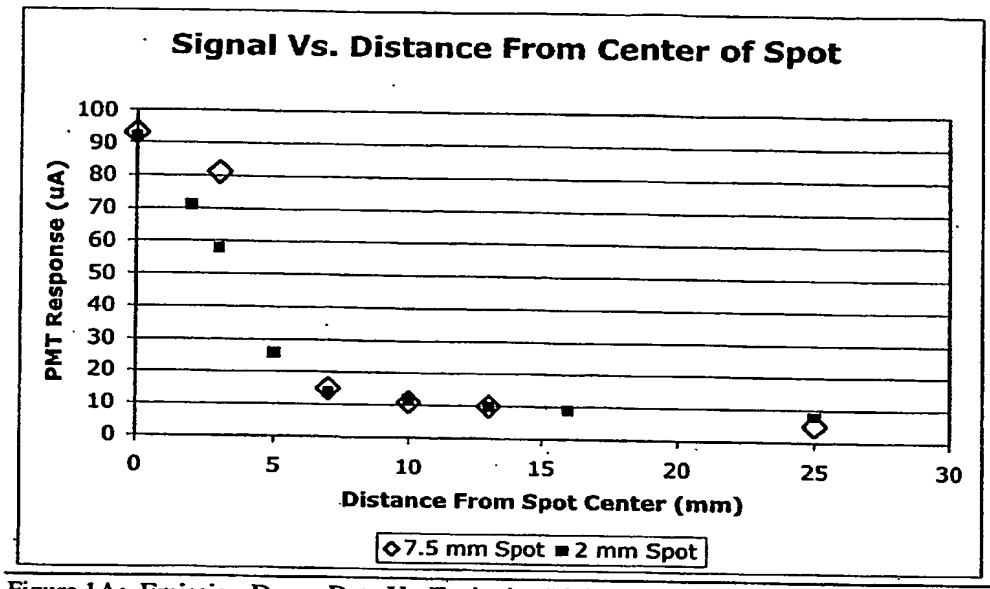


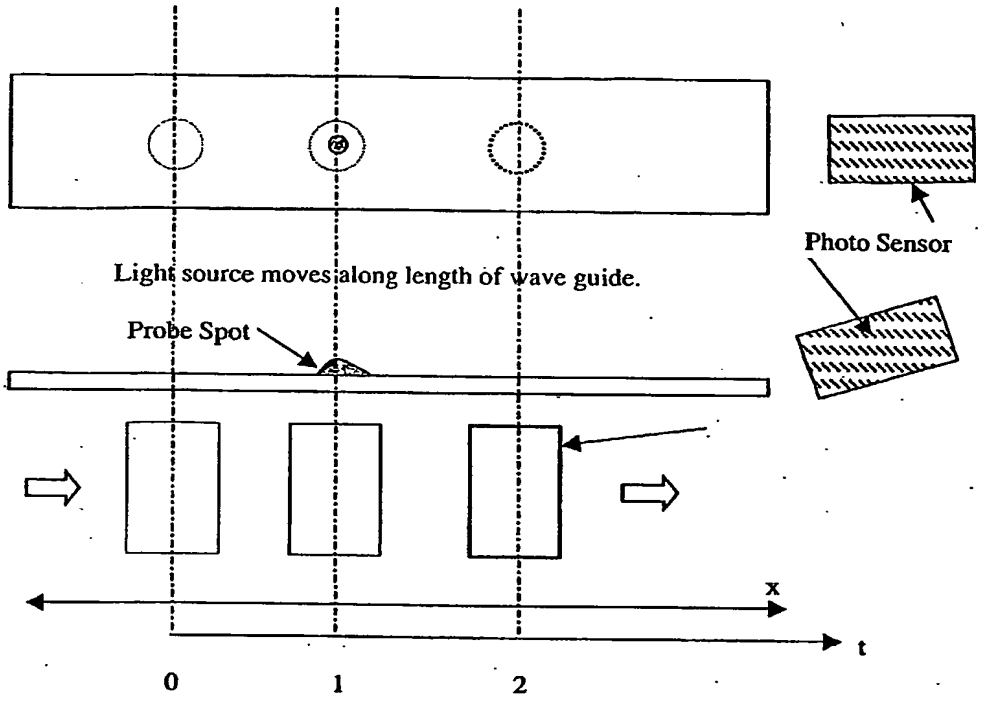
Figure 1A: Emission Decay Data Vs. Excitation Light Alignment

Figure 1A shows the emission data collected from a test device where the light source was moved relative to the center of a single fluorescent spot immobilized on a 25mm x 75mm x 1mm glass planar waveguide. Readings were taken from a photo detector aligned to one end of the planar wave guide as shown in Figure 1 of the original document. Subsequent readings were taken as the light source was advanced in the long direction of the wave guide away from the spot. The result as depicted in the figure, demonstrates a decay that is related to the square of the distance between the light source center and the probe spot center.

Signal Reading As Detection

The utility of this decaying relationship between emission and alignment to excitation is that it enables a means to discriminate between multiple spots on the same waveguide surface. It further enables a means to discriminate between spots drawn close enough together that they fluoresce simultaneously. These means are explained in here.

Addendum 1



Data Collection:
simultaneous collection of light position and photo sensor output.

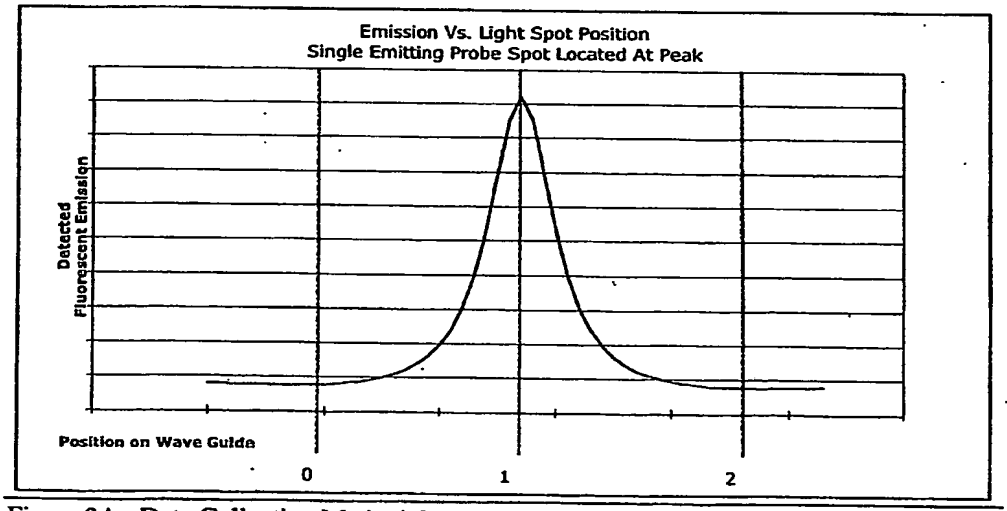


Figure 2A: Data Collection Method & Projected Readings

Figure 2A demonstrates that if a light source is advanced along a known direction relative to a fluorescently excitable spot, the photo sensor output varies as a function of the position of the light source relative to the planar waveguide. Figure 2A shows the output from one spot on a surface. Figure 3A shows the output associated with a similar illuminating sweep taken over a surface with two spots. Each of the dotted lines shows the reading that would be taken were only one or the other of the two spots fluorescently hybridized. The heavy solid line depicts the signal that would be generated were both spots fluorescently hybridized. At the spacing shown here, both spots would be distinguishable from one another regardless of their respective fluorescent states. A spacing producing such an output could yield distinguishable results by taking discrete measurements, positioning the light source at the loci coincident with the probe spot centers, p1 and p2.

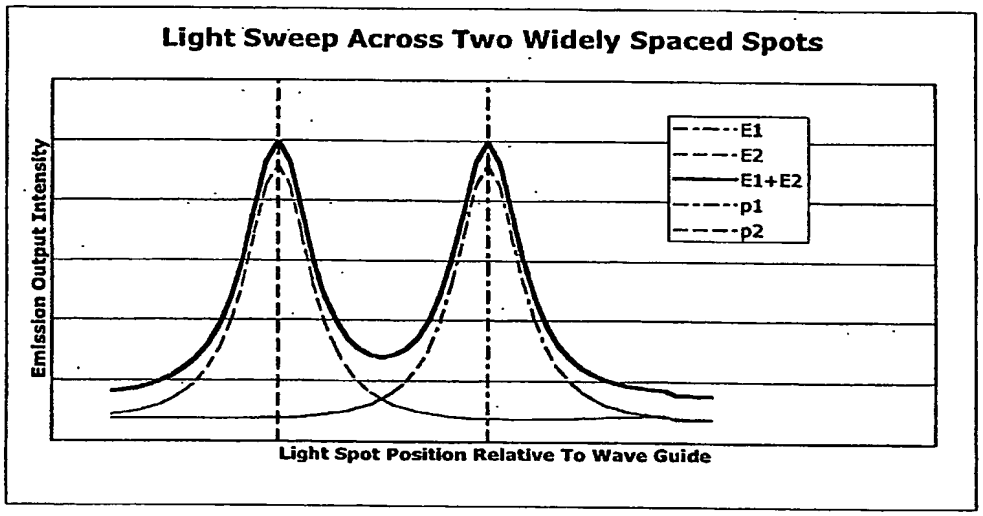


Figure 3A: Two Spot Sweep Data, Wide Spacing

Were the probe spots brought closer together, the combined signals would become more conglomerated, and thus more difficult to discretely distinguish from one another, Figure 4A. In this spacing, which would accommodate a higher number of probe spots (i.e. test sites), discrete measurements would fail to provide unambiguous results. It would not be clear if the signal read at position 1 comes from fluorescence of spot 1 or spot 2 e.g. However, were a linearly continuous measurement taken, yielding an emission signature as depicted in either Figures 3A or 4A, any of the possible combinations of fluorescent states among the two probe spots can be distinguished. In Figure 4A, the signature illustrating the case where both spots are fluorescent, is distinguishable from the signature of either or neither of the spots fluorescing individually. This same concept can be expanded to inspect linear arrays of spots that are more numerous than 2. It is

conceivable, based on current evidence collected that 5 or more spots could be detected in a linear space of approximately 25 mm.

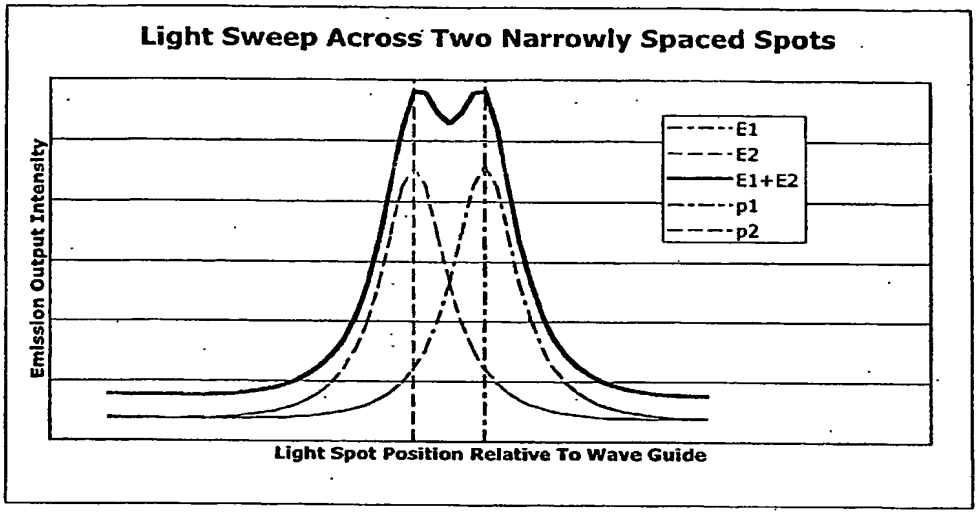


Figure 4A: Two Spot Sweep Data, Narrow Spacing

Conclusion

In summary, the conceptual framework for multiplexed detection on a single planar wave guide surface has been established. It consists of a process of moving a single excitation light source in a known trajectory relative to the planar wave guide. Placed upon the planar waveguide are a series of probe spots whose individual fluorescent states are unknown, yet whose loci are well established. Simultaneous and continuous measurements are taken of light source position along the wave guide and the intensity of emission light detected by the photo sensor located at one of the wave guide's ends. The resulting data, like that shown in Figures 2A, 3A, & 4A demonstrates that relatively closely packed spots can be distinguished, even when their individual signals interfere with one another. Since the intensity vs. light spot position signatures of combined spots are different from the signatures generated from single spots, the distinction between spots is achievable.

Introduction

This document is the second addendum to the disclosure of pages A1-A-10, referred to herein as the original document. Following the embodiments discussed in Addendum 1, discussed here is a means to further the utility of the described invention. Primarily focused here is the demonstration of comparing the relative intensities of fluorescently labeled spots co-located on a single wave guide surface. Determination of relative intensity broadens the application of this invention into aiding investigation of levels of relative target in addition to presence of target previously discussed.

In general, given the same reading method described in Addendum 1, multiple spots of different fluorescence intensity, can be distinguished from one another, and their relative intensity, one compared to the other, can be inferred. Figures 2A and 3A of Addendum 1, show the projected photo sensor response to 2 spots spaced at different distance from one another. Figure 1A2 shown below in this document shows the projected photo detector response to two spots where spot 1 produces half the maximum intensity of spot 2. As discussed in Addendum 1, the solid line shows that when a continuous / quasi-continuous measurement is taken, the two spots are distinguishable. Furthermore, their relative intensities can be determined by analyzing the difference in peak height as well as the location and relative magnitude of the inflection point between them.

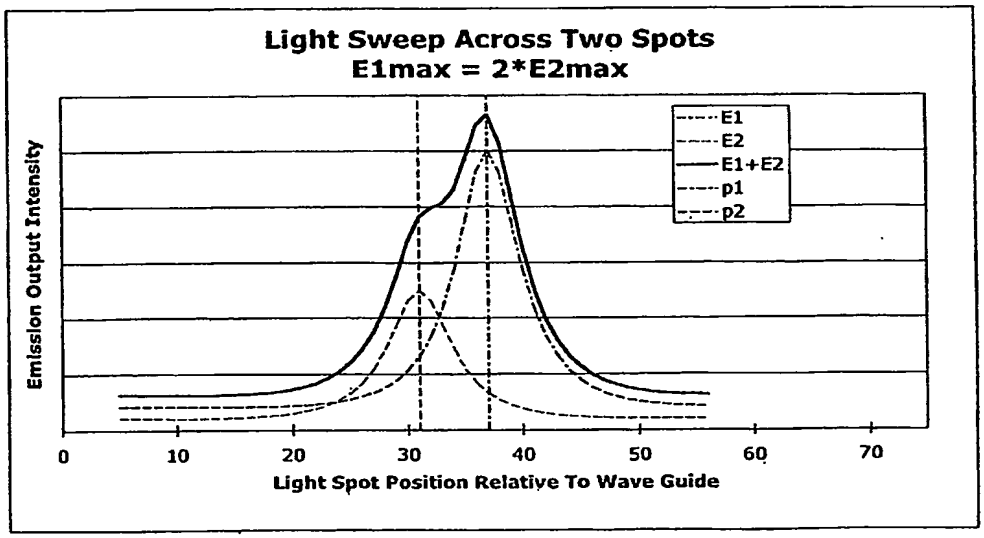
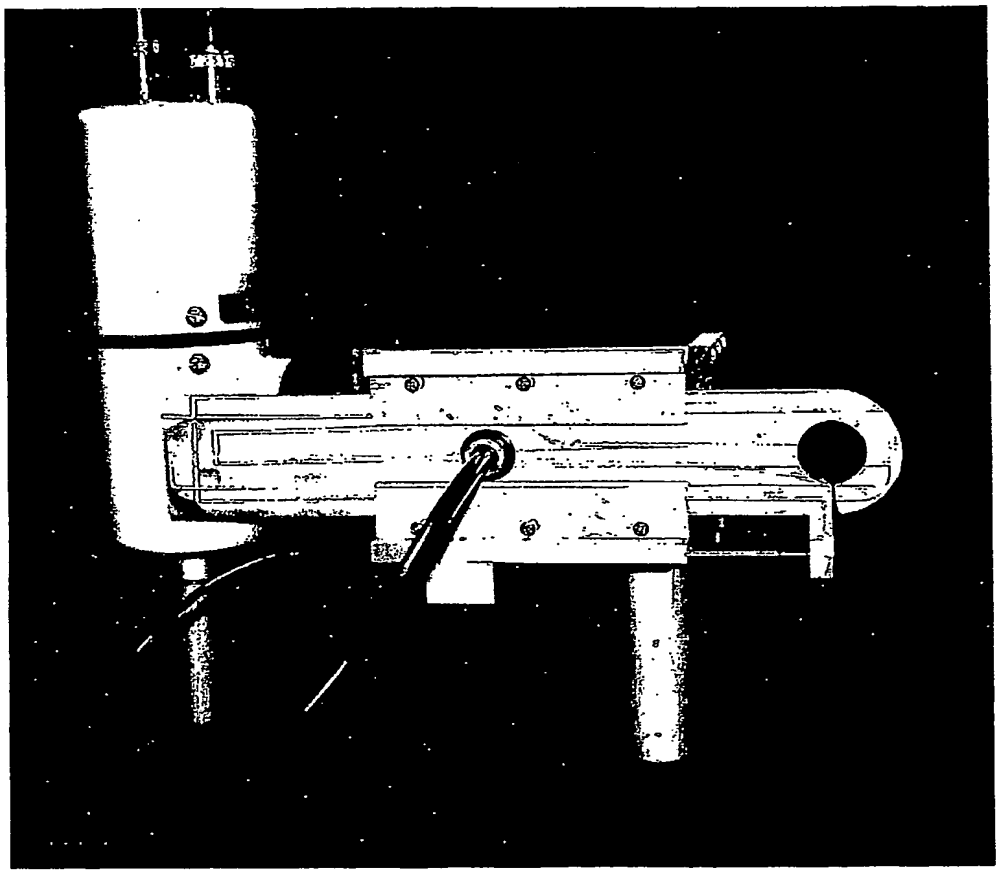
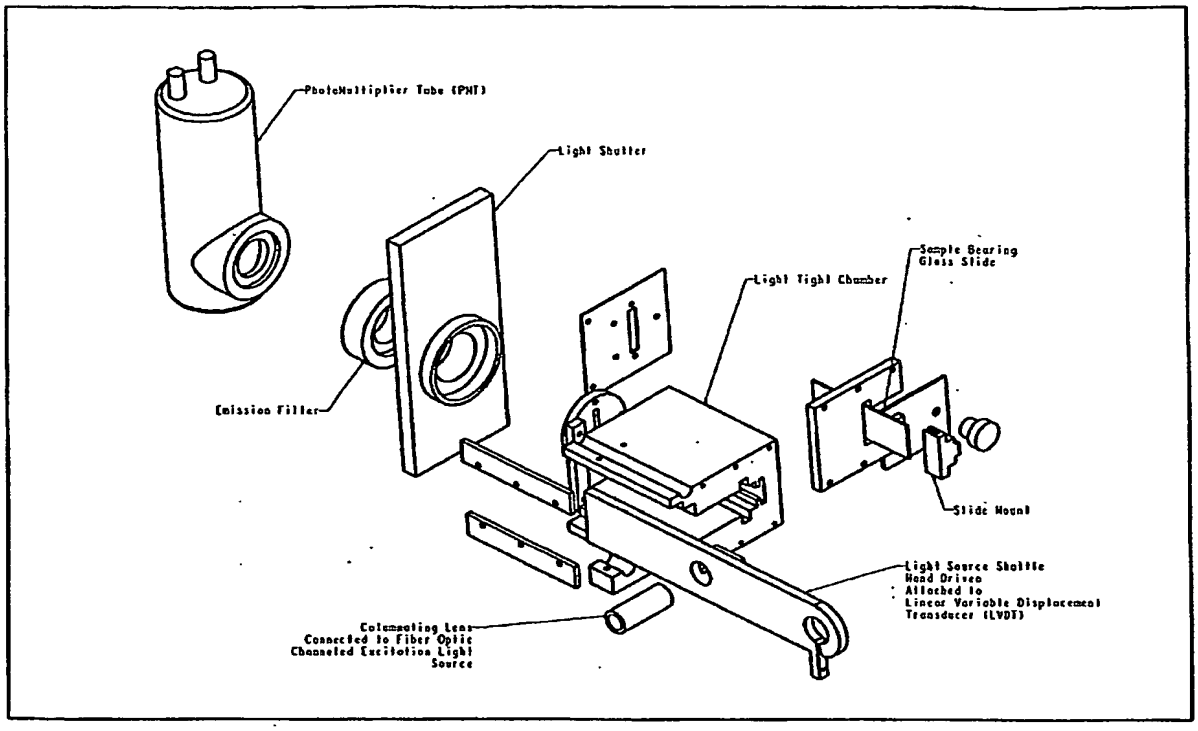


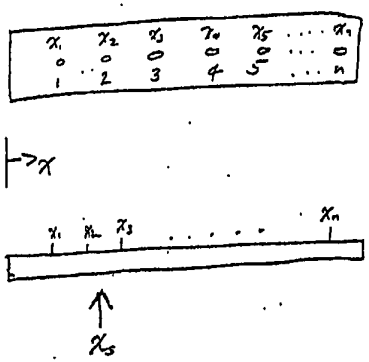
Figure 1A2



A-17



- AN EXPLODED DIAGRAM OF THE SYSTEM DEPICTED IN THE PHOTOGRAPH ON PAGE A-17



x_i = X pos of test site i

x_s = X pos of excitation light.

Assume net emission from all spots @ a given x_s is a linear superposition of individual spot emissions.

$$\Rightarrow E = \sum_{i=1}^{n} E_i$$

$E_i = E_i^0 \cdot A(x_s, x_i) \longrightarrow E_i^0 \equiv$ emission of spot i where $x_s = x_i$

Preliminary data suggests

$$A(x_s, x_i) = 1 - \left[\frac{(x_s - x_i)^2}{\beta + (x_s - x_i)^2} + \frac{\text{abs}(x_s - x_i)}{\alpha + \text{abs}(x_s - x_i)}\right]$$

$$\Rightarrow E(x_s) = \sum_{i=1}^{n} E_i^0 A(x_s, x_i)$$

$E(x_s)$ – Quantity measured continuously over x_s by PWG

$x_1, x_2, x_3 \ldots x_n$ – Known because test site locations are known

x_s – known as measured value from PWG

E_i^0 – unknown

Solving For E_n^0

Method: Solve n coupled linear equations simultaneously
n = # of test sites on wave guide

[1] Take continuous sweep measurement with PWG

Result

x_s	$E(x_s)$
—	—
—	—
—	—
⋮	⋮

=>

[2] Take n points from [1], set up n equations for E

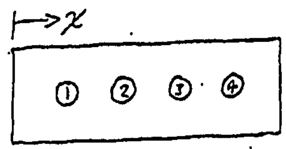
$$E(x_{s1}) = E_1^0 A(x_{s1}, x_1) + E_2^0 A(x_{s1}, x_2) + \cdots + E_n^0 A(x_{s1}, x_n)$$
$$E(x_{s2}) = E_1^0 A(x_{s2}, x_1) + E_2^0 A(x_{s2}, x_2) + \cdots + E_n^0 A(x_{s2}, x_n)$$
$$\vdots$$
$$E(x_{sn}) = E_1^0 A(x_{sn}, x_1) + E_2^0 A(x_{sn}, x_2) + \cdots + E_n^0 (x_{sn}, x_n)$$

⇓

$$[A]\{E^0\} = \{E\}$$

Solution: $\{E^0\} = [A]^{-1}\{E\}$

EXAMPLE



1 - positive control $x_1 = 10$

2 - wild type $x_2 = 15$

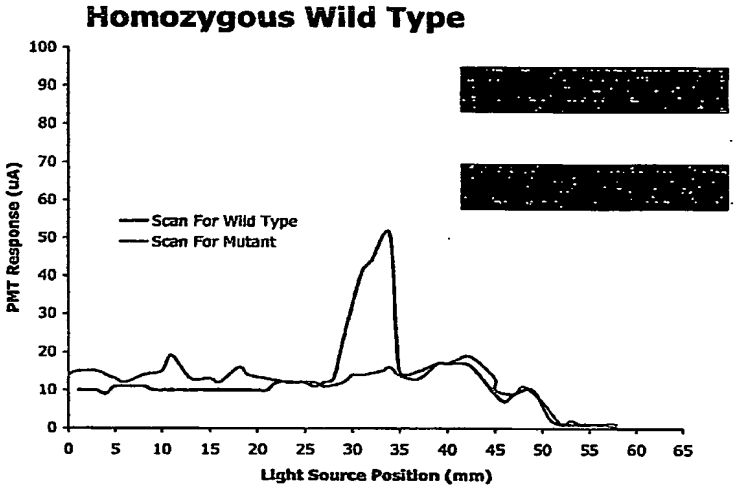
3 - mutant $x_3 = 20$

4 - negative control $x_4 = 25$

#	x_s	E
1.	10	63.6
2	15	53.1
3	20	61.7
4	25.	32.3

Note: E values are simulated

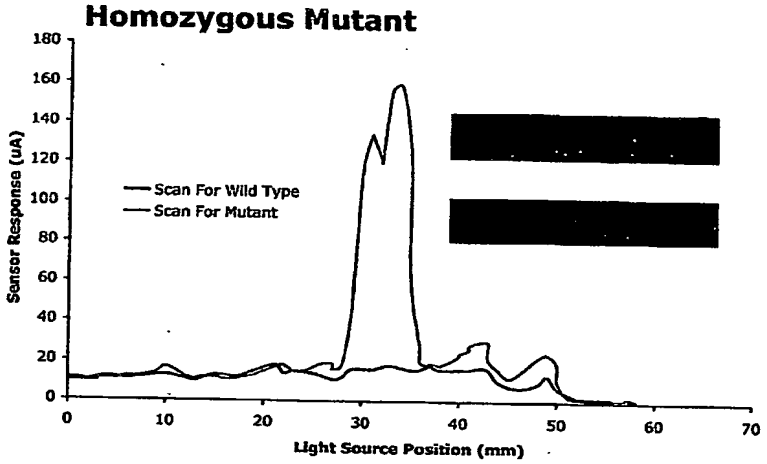
1. The plots shown here are collected using a prototype developed in house that follows the reading process laid out in other disclosure documents. In general, a light source moves over the length of a slide while a photo sensor continuously records florescent emission from one of the slides ends.
2. The images shown along with each of the plots are those of the slides scanned. Images were generated from a microarray slide laser scanner.



Factor V Leiden Clinical Sample: Homozygous Wild Type

Two different slides were prepared, one with probes for the mutant sequence, the other with probes for the wild type. Note the mutant slide image is dark and no peak is present in the device data. Similarly, the wild type slide has a fluorescent spot, and the device data shows a significant peak at the location of the spot.

[redacted]



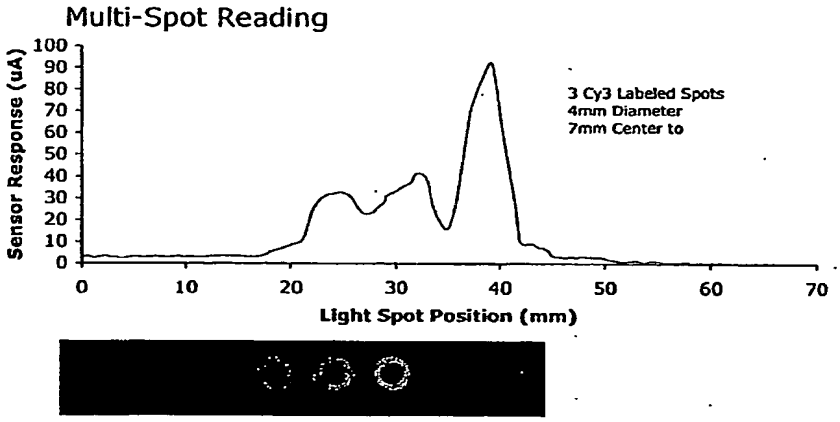
Factor V Leiden Clinical Sample: Homozygous Mutant

Two different slides were prepared, one with probes for the mutant sequence, the other with probes for the wild type. Note the wild type slide image is dark and no peak is present in the device data. Similarly, the mutant slide has a fluorescent spot, and the device data shows a significant peak at the location of the spot.

	Clinical		Laser Scanner		PWG Device	
Spec. No.	Wild Type	Mutant	Wild Type	Mutant	Wild Type	Mutant
1	+	-	+	-	+	-
2	+	-	+	-	[redacted]	-
3	+	-	+	-	+	-
4	+	-	+	-	+	-
5	+	-	+	-	+	-
6	+	-	+	-	+	-
7	+	-	+	-	+	-
8	+	-	+	-	+	-
9	+	-	+	-	+	-
10	+	-	+	-	+	-
11	+	-	+	-	+	-
12	+	-	+	-	+	-
13	+	-	+	-	+	-
14	+	-	+	-	+	-
15	+	-	+	-	+	-
16	+	-	+	-	+	-
17	+	-	+	-	+	-
18	-	+	-	+	-	+
19	-	+	-	+	-	+
20	-	+	-	+	-	+
21	-	+	-	+	-	+

Table: 21 Patients Tested
Shows consistency between PWG device on other detection means 98% Correlation

[redacted]



Three Spot Scan on the Same Slide.

Note from the laser scanner picture of the slide, the 3rd spot from the left is the brightest. The signal detected by our device reflects its greater brightness by recording a higher peak. The system provides a quantitative means to compare the intensities of different spots. This figure shows that not only can the invention described here distinguish between multiple spots on the same substrate, but also compare their relative intensities.

1.
A fluorescent spot detection system comprising an optical wave guide, excitation light source, and photo detector. The excitation light source moves relative to the wave guide over a known interval or advancement velocity. Simultaneously, output from the photo detector (statically positioned at one of the wave guide's ends) is continuously or quasi-continuously collected and correlated with excitation light position. Spot detection is achieved through characterization of photo detector response relative to excitation light position.

2.
A means to discern between fluorescent spots co-located on the same wave guide surface whose spacing is close enough to cause overlap of fluorescent emission signal. This means is the de-convolution of data (described in 1) taken across the entire field on the wave guide of possible spot loci. De-convolution is based on the prior characterization of known fluorescent spot arrangements.

3.
A means to discern relative intensity between fluorescent spots co-located on the same wave guide surface whose spacing may or may not cause overlap of fluorescent emission signal. This means is the de-convolution of data (described in 1) taken across the entire field on the wave guide of possible spot loci. De-convolution is based on the prior characterization of known fluorescent spot arrangements with known relative intensities.

4.
A fluorescent, diffraction, or otherwise light interfering/absorbing spot detection system comprising an optical wave guide, illumination light source, and photo detector. The light source (held static relative to the waveguide) provides uniform illumination over the region of the wave guide where spots are potentially located. The photo detector moves relative to the wave guide over a known interval or advancement velocity. Output from the photo detector is continuously or quasi-continuously collected and correlated with the photo detector's position relative to the wave guide. Spot detection is achieved through characterization of photo detector response relative to photo detector position.

5.
A means to discern between spots (described in 4) co-located on the same wave guide surface whose spacing is close enough to cause overlap of their respective individual patterns of interference/absorption. This means is the de-convolution of data (described in 4) taken across the entire field on the wave guide of possible spot loci. De-convolution is based on the prior characterization of known spot arrangements.

6.
A means to discern relative interference/absorption between spots (described in 4) co-located on the same wave guide surface whose spacing may or may not cause overlap of light interference/absorption. This means is the de-convolution of data (described in 4) taken across the entire field on the wave guide of possible spot loci. De-convolution is

based on the prior characterization of known interference/absorption spot arrangements with known relative interference/absorption characteristics.

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"Express Mail" mailing label number: EV 183608488 US
Date of Deposit: November 13, 2002

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR §1.10 on the date indicated above and is addressed to the Commissioner for Patents, ATTN: Box Provisional Application, Washington, D.C. 20231.

By: Sue Dombroske
Name: Sue Dombroske